Molecular and Cellular Mechanisms of Asbestos Fiber Toxicity

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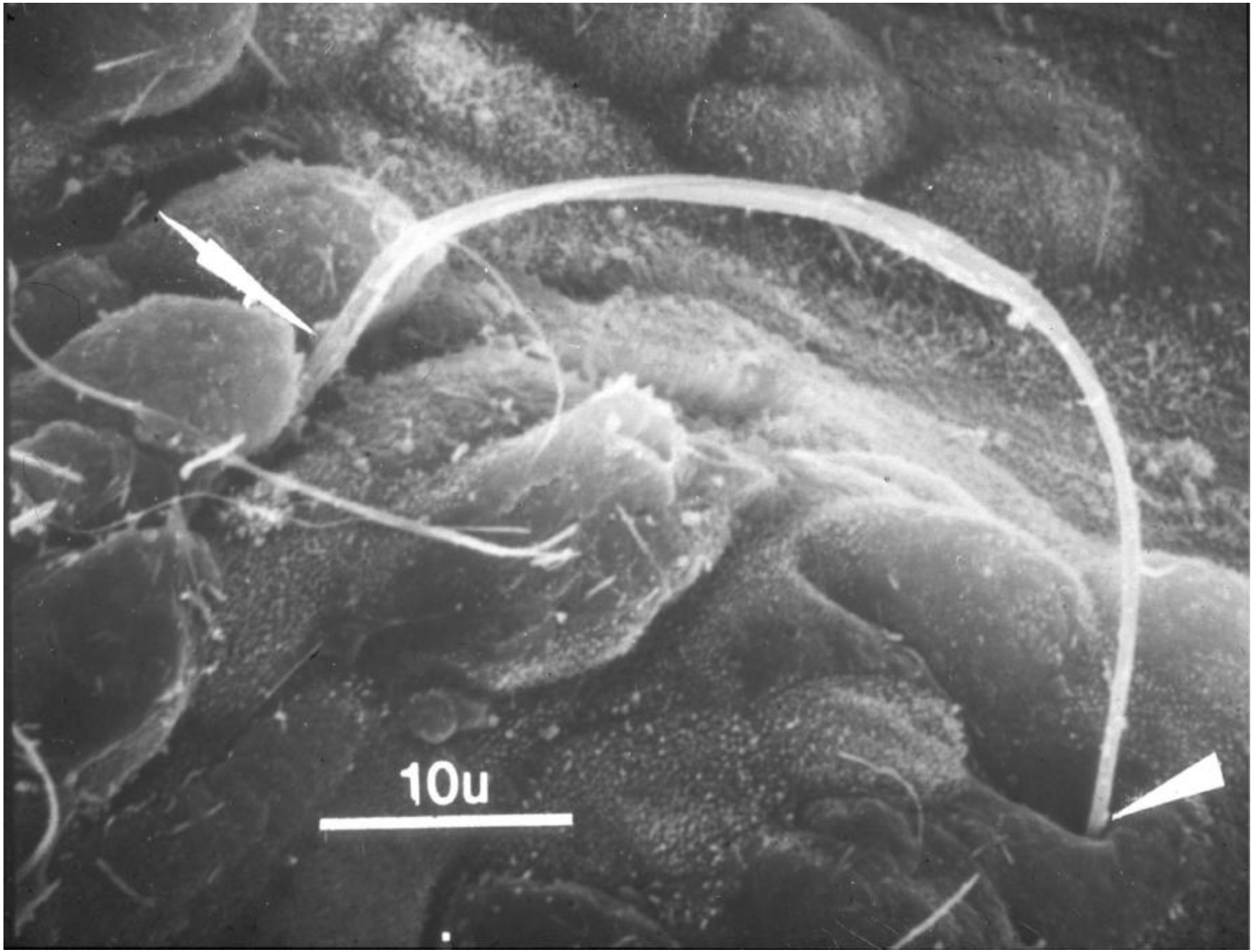
Cellular and Molecular Mechanisms of Asbestos Fiber Toxicity

- Interaction of asbestos fibers with cells of the respiratory tract
  - chemical and physical properties of fibers
  - iron-catalyzed reactions that generate reactive oxygen species (ROS)
- Sequence of cellular events after inhalation of asbestos fibers
  - inflammatory response
  - production of cytokines/chemokines/growth factors
  - cell injury and proliferation

Mechanisms cont.

- **Sequence of cell signaling events stimulated by asbestos fibers**
  - **signal transduction pathways [e.g., Mitogen Activated Protein Kinase (MAPK) pathway]**
  - **activation of transcription factors [e.g., Activator Protein-1 (AP-1)]**
  - **alterations in gene expression**
  - **cell injury, survival, proliferation, transformation**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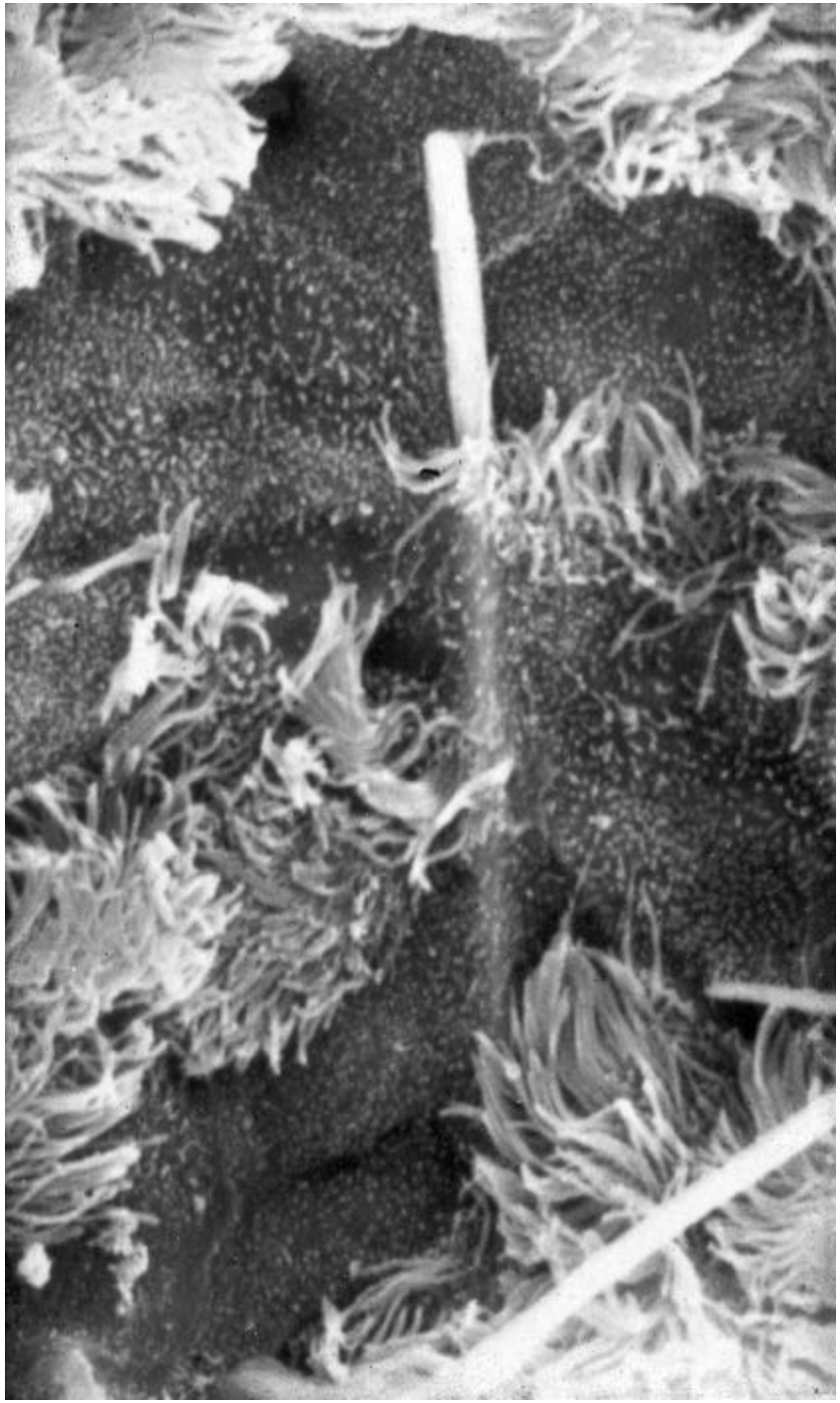
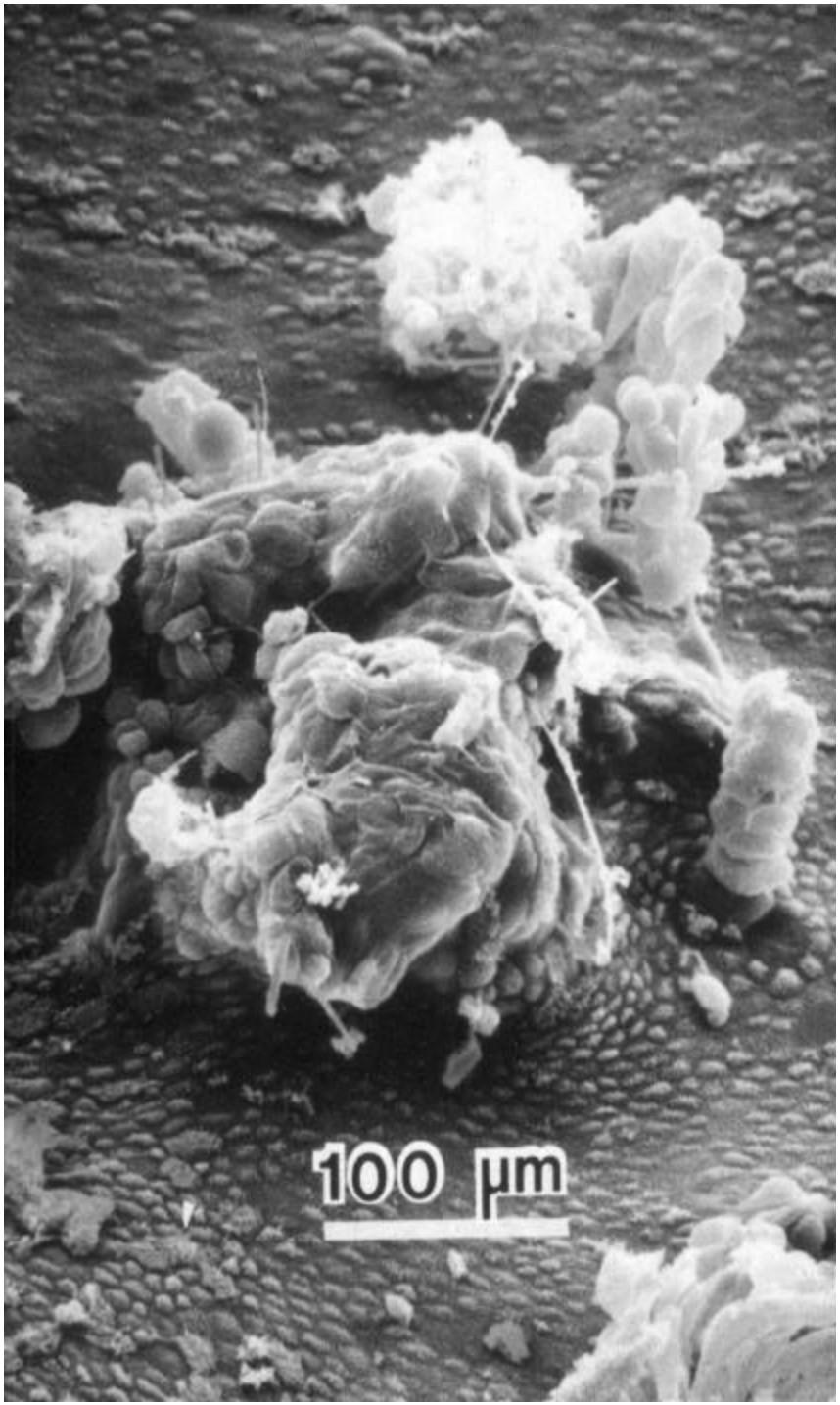
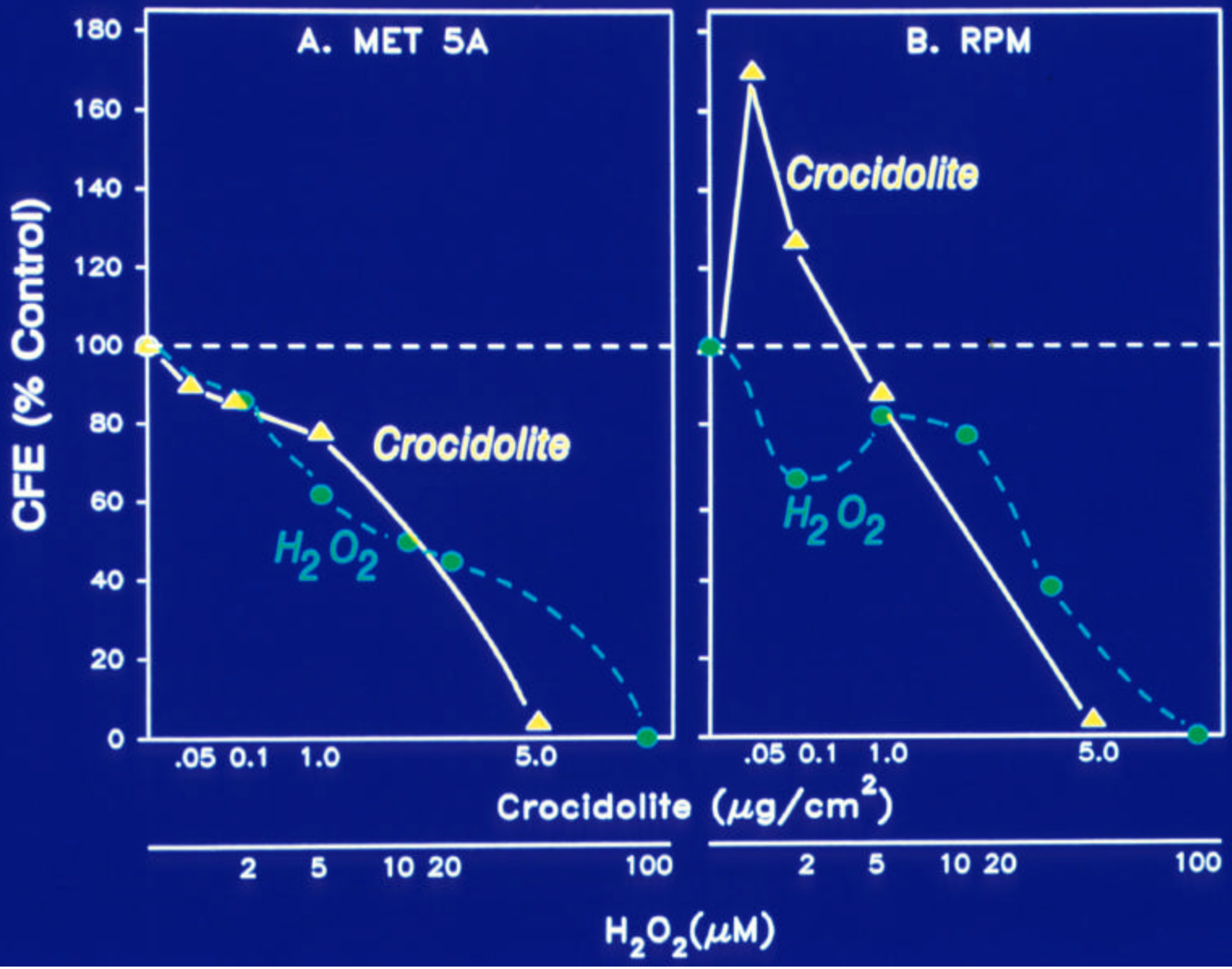
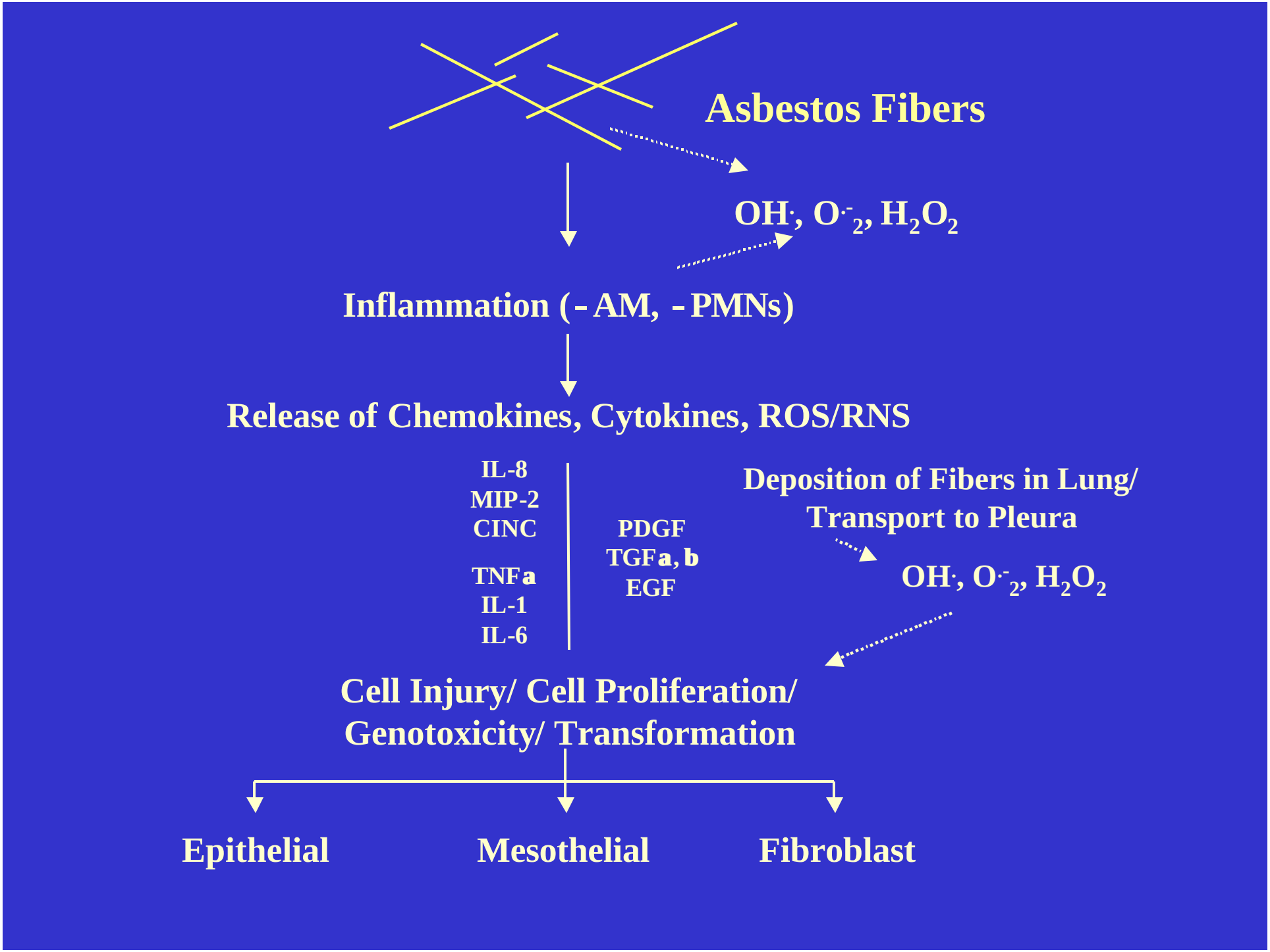



100 µm

COLONY FORMING ABILITY OF HUMAN MET 5A CELLS AND RAT PLEURAL MESOTHELIAL (RPM) CELLS FOLLOWING EXPOSURE TO ASBESTOS




Asbestos Fibers
OH·, O·-2, H2O2
Inflammation (- AM, - PMNs)
Release of Chemokines, Cytokines, ROS/RNS
IL-8
MIP-2
CINC
TNFa
IL-1
IL-6
PDGF
TGFa, b
EGF
Deposition of Fibers in Lung/
Transport to Pleura
OH·, O·-2, H2O2
Cell Injury/ Cell Proliferation/
Genotoxicity/ Transformation
Epithelial
Mesothelial
Fibroblast

Cell Signaling → **Transcription Factor Activation** →

(MAPK)

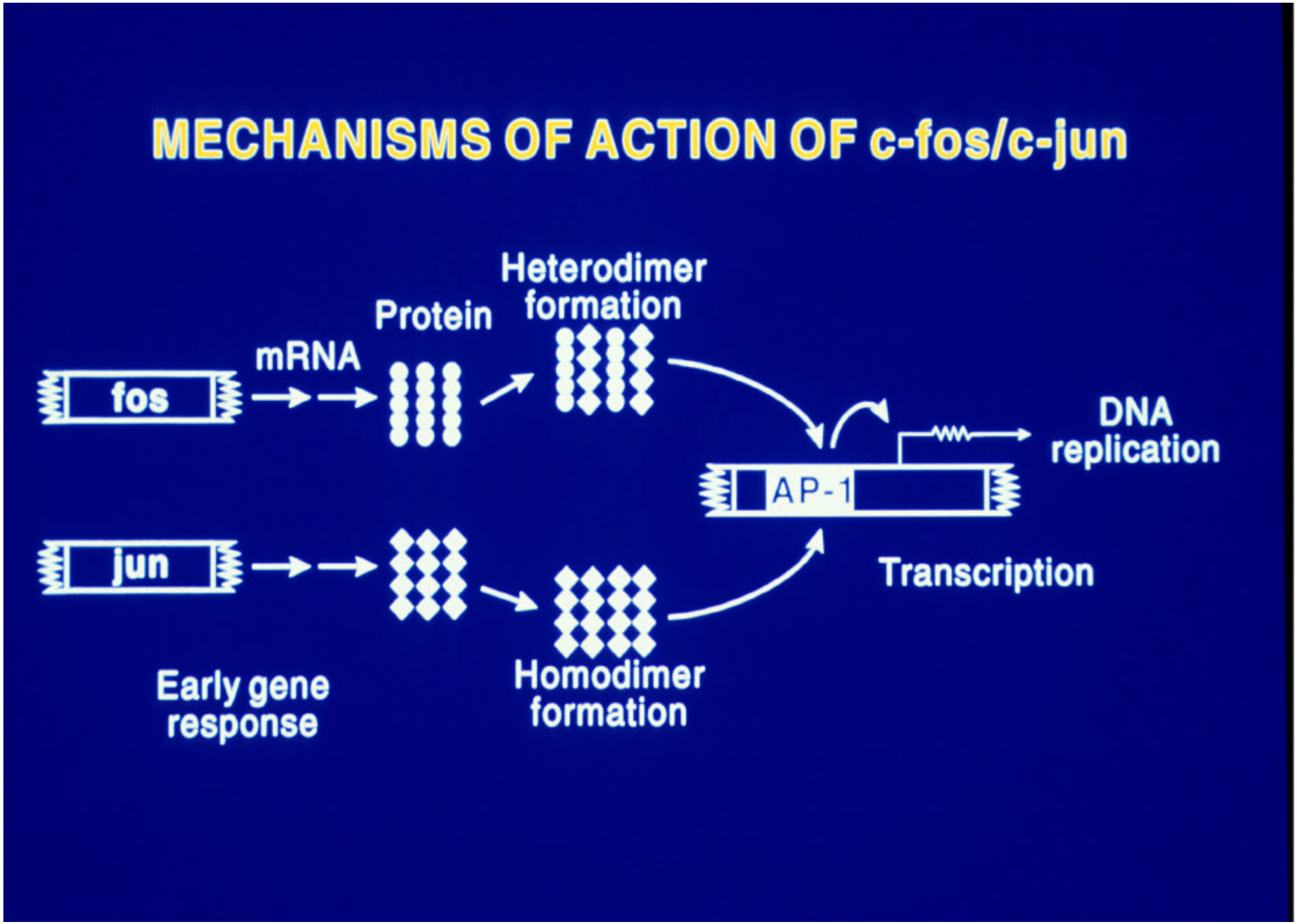
(AP-1)

Gene Expression → **Biological Response** → **Disease**

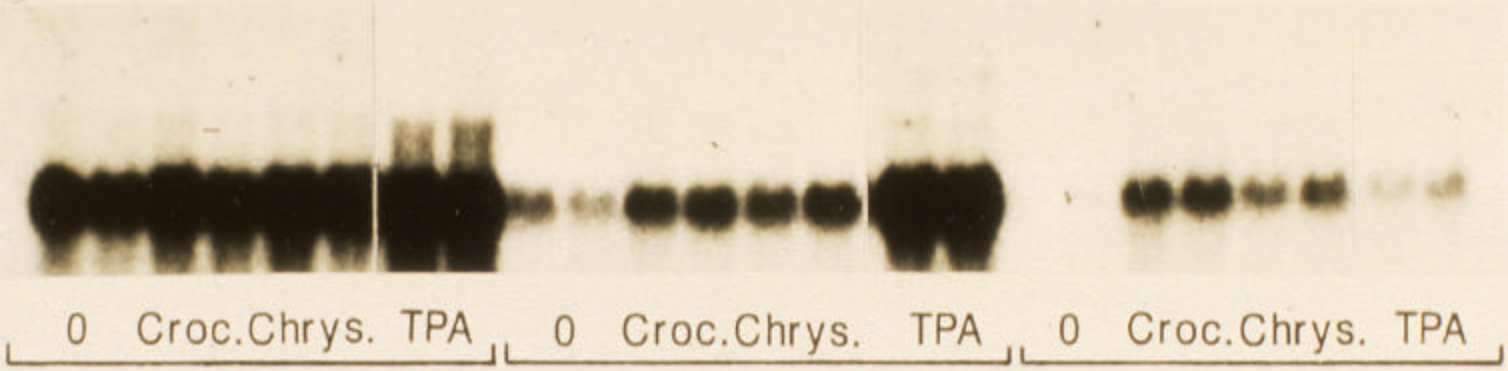
Cell Injury/Survival

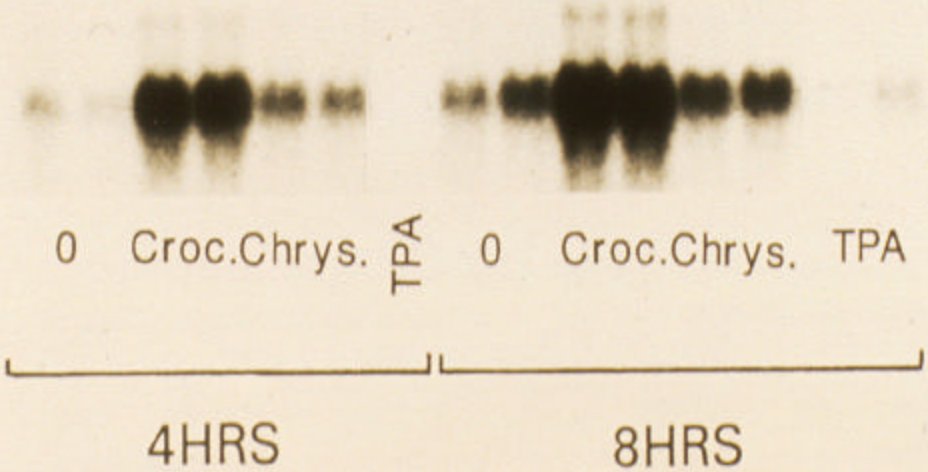
Cell Proliferation

Cell Transformation

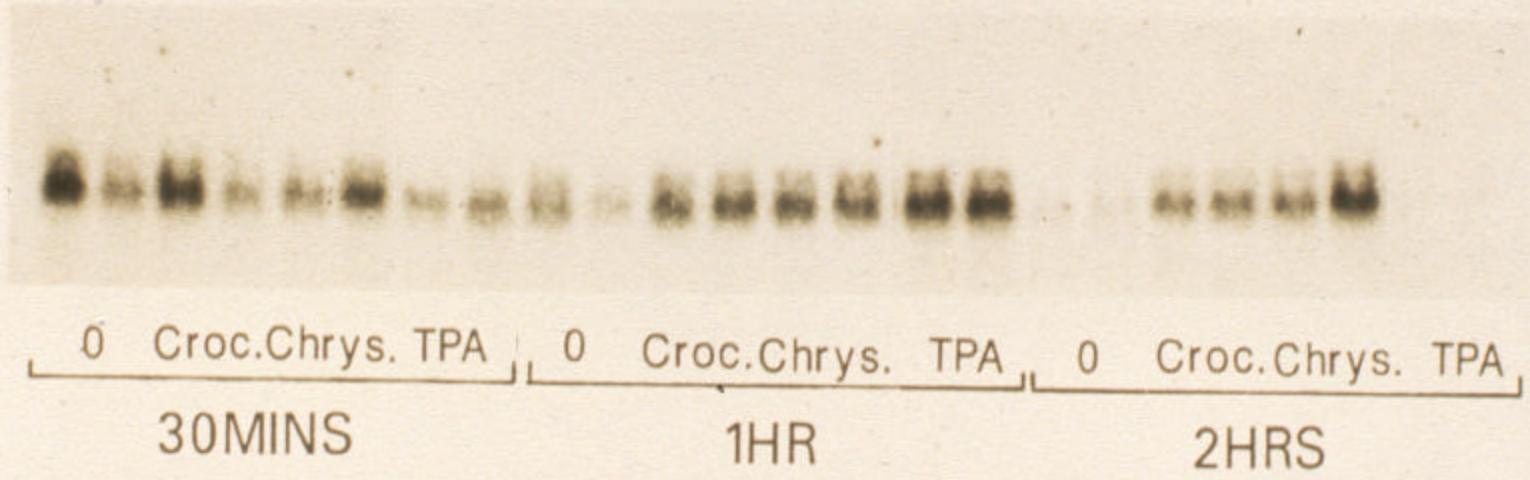

MECHANISMS OF ACTION OF c-fos/c-jun
Heterodimer formation
Protein
mRNA
fos
DNA replication
AP-1
jun
Transcription
Early gene response
Homodimer formation

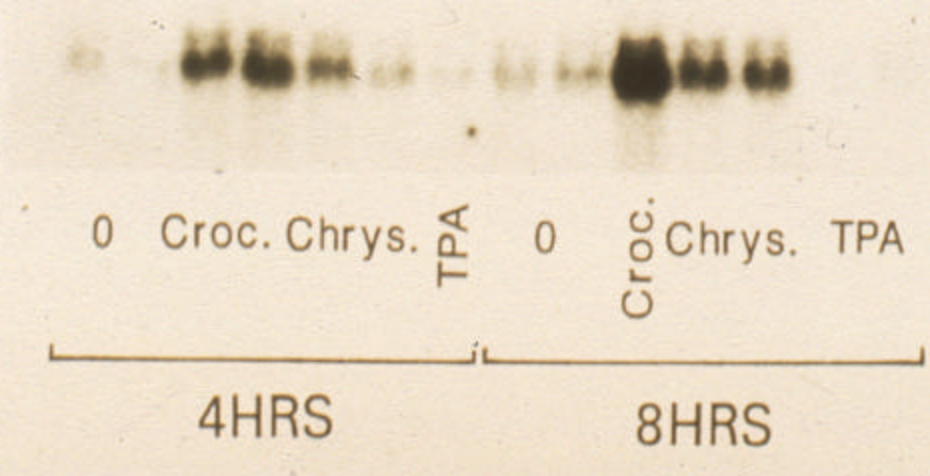
Steady-state mRNA Levels of c-fos in Rat Pleural Mesothelial Cells (RPM)



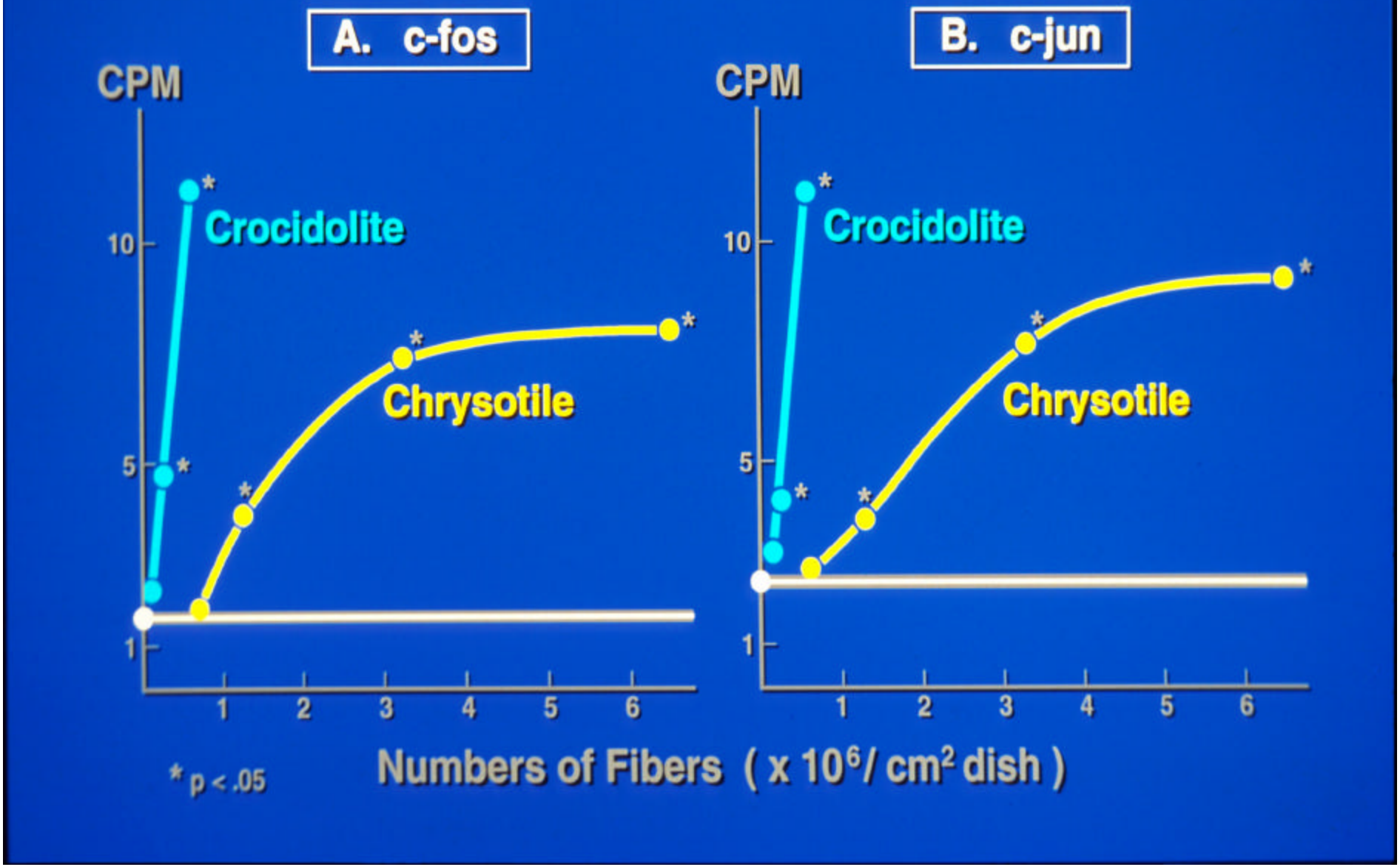


Steady-state mRNA Levels of c-jun in Rat Pleural Mesothelial Cells (RPM)

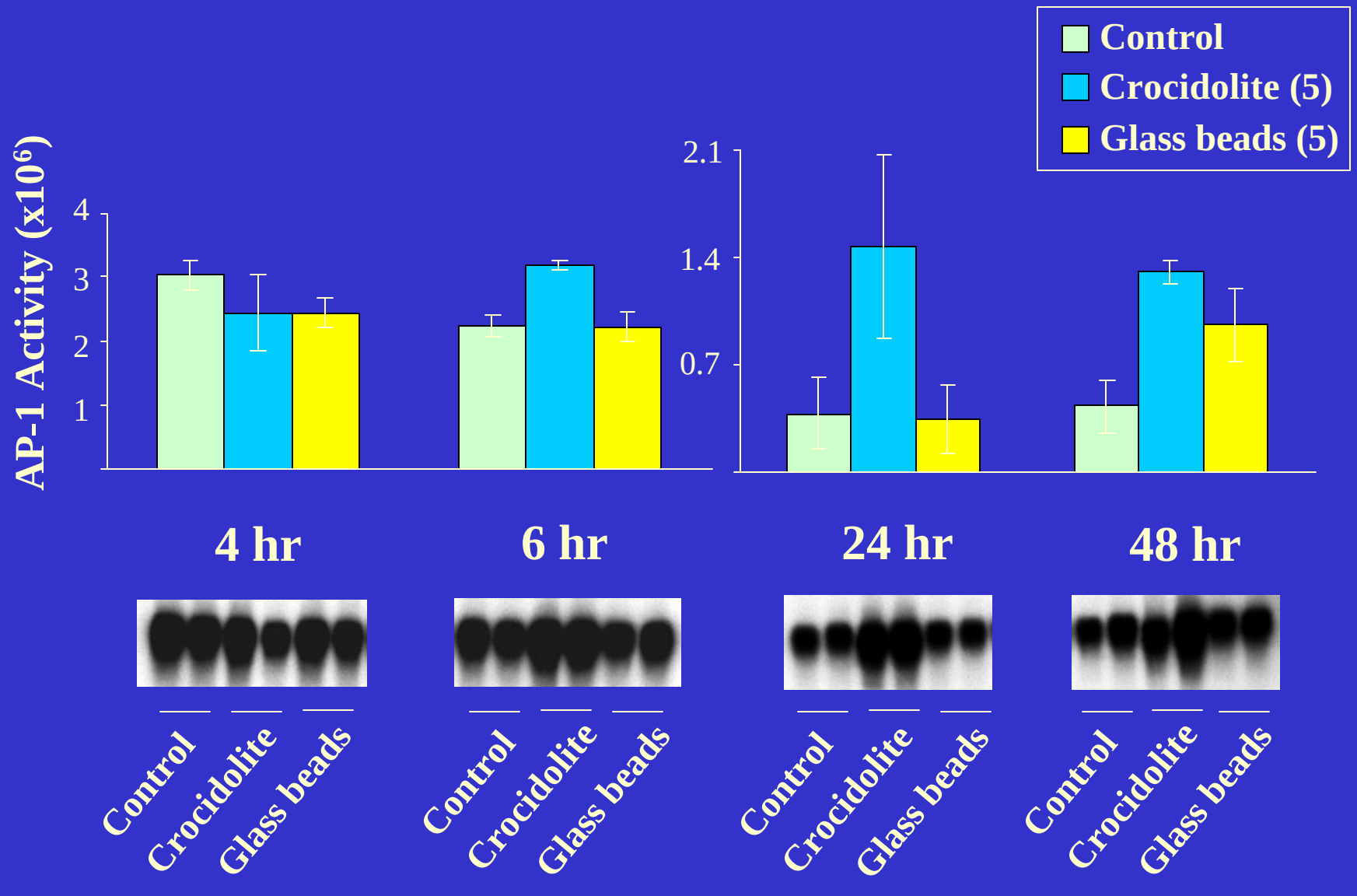


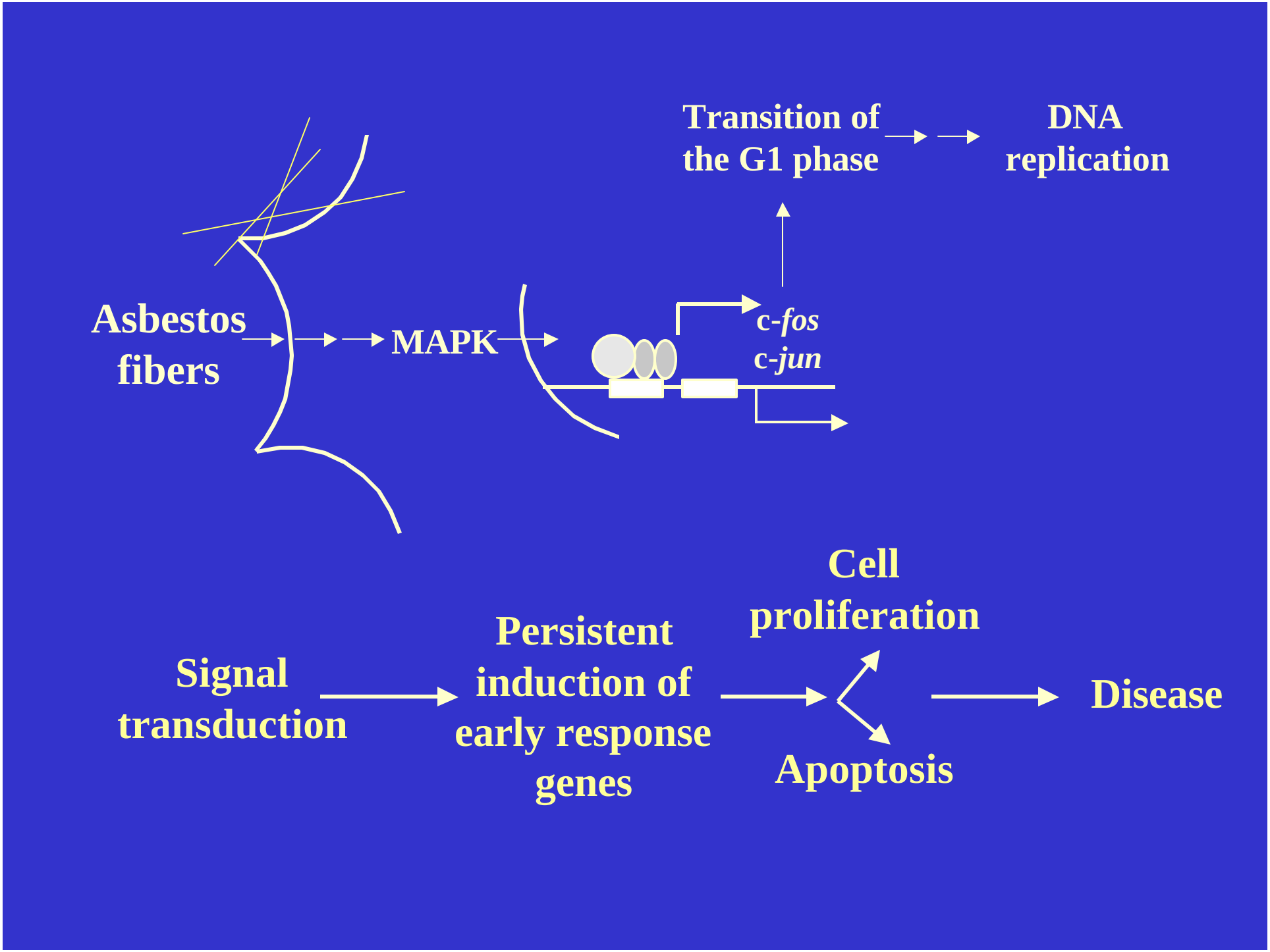


GENE EXPRESSION OF PROTOONCOGENES AFTER EXPOSURE OF RAT PLEURAL MESOTHELIAL (RPM) CELLS TO ASBESTOS



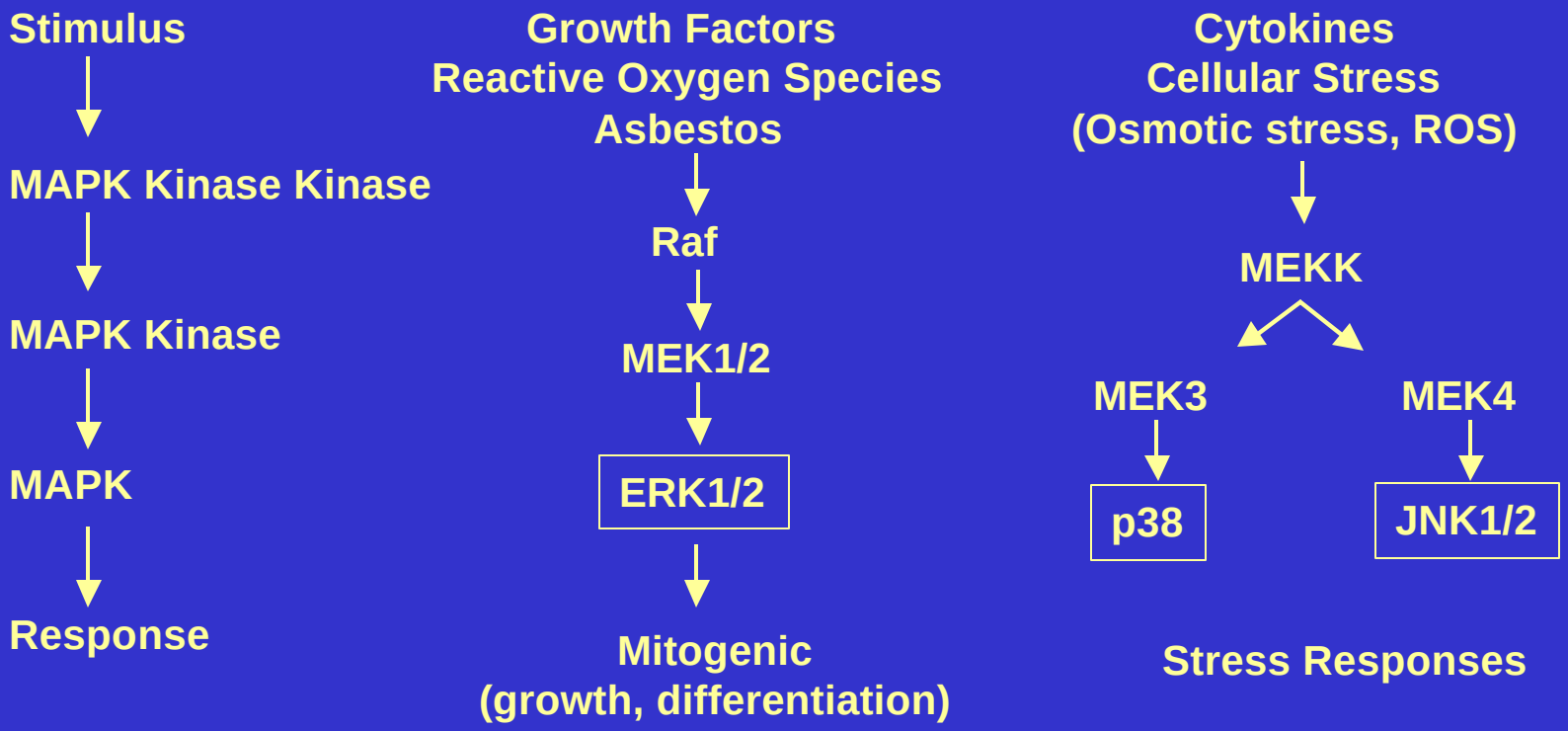
Asbestos-Induced AP-1 Activity in Mesothelial Cells



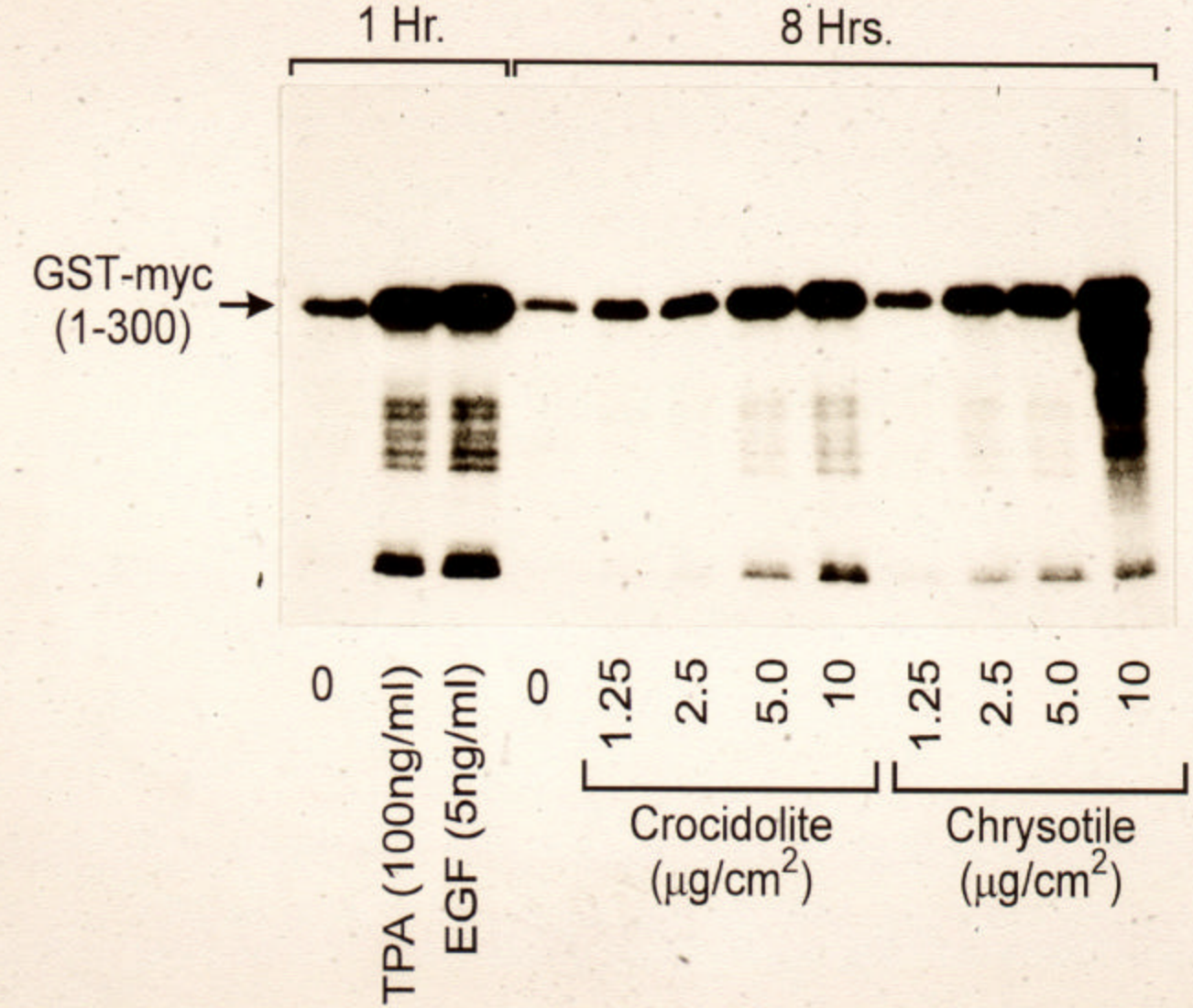

Transition of the G1 phase
DNA replication
Asbestos fibers
MAPK
c-fos
c-jun
Signal transduction
Persistent induction of early response genes
Cell proliferation
Apoptosis
Disease

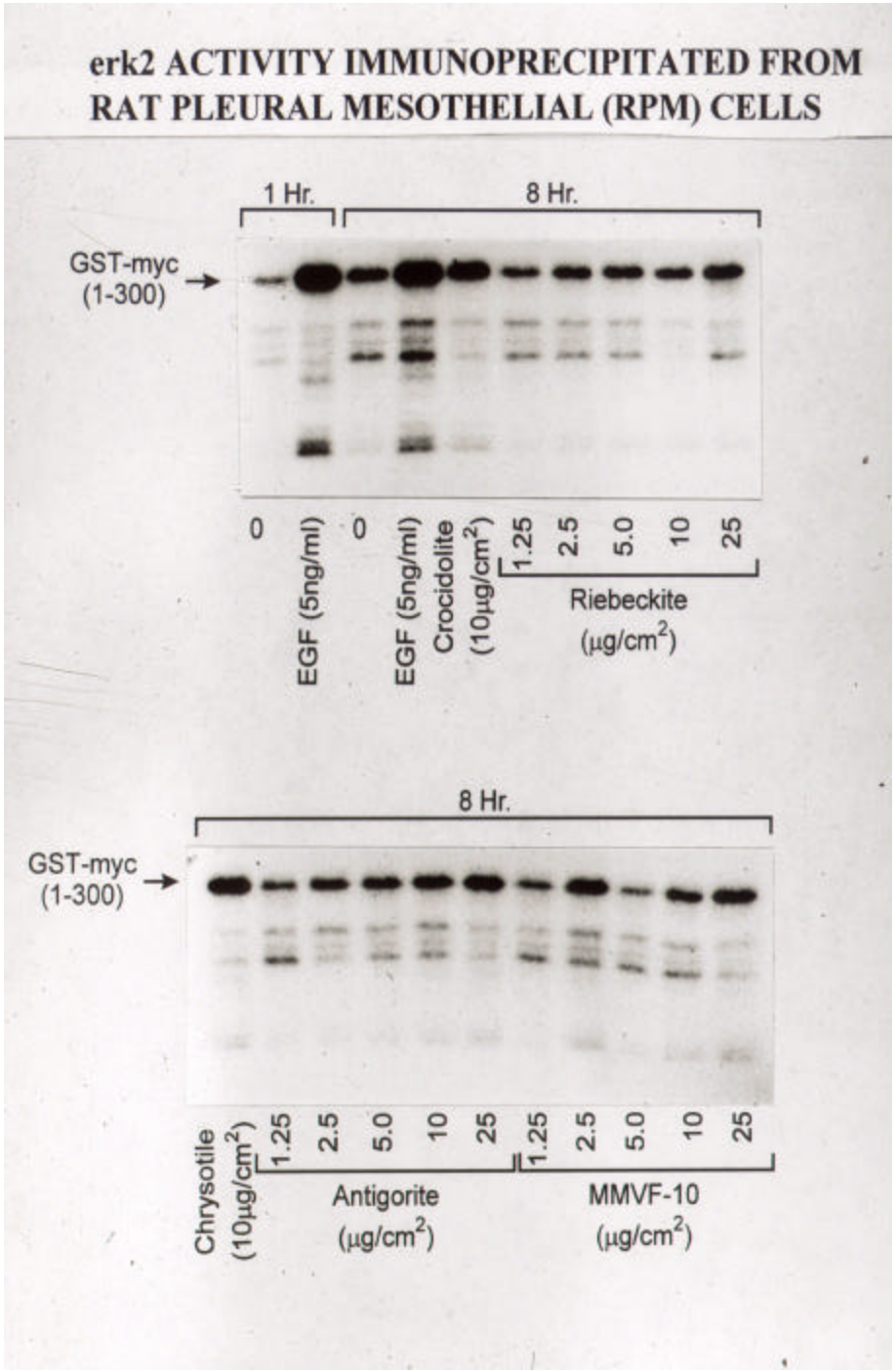
MITOGEN-ACTIVATED PROTEIN KINASE (MAPK) CASCADE

* ERK=Extracellular Signal-Regulated Kinase
* JNK=c-Jun N-terminal Kinase
* p38

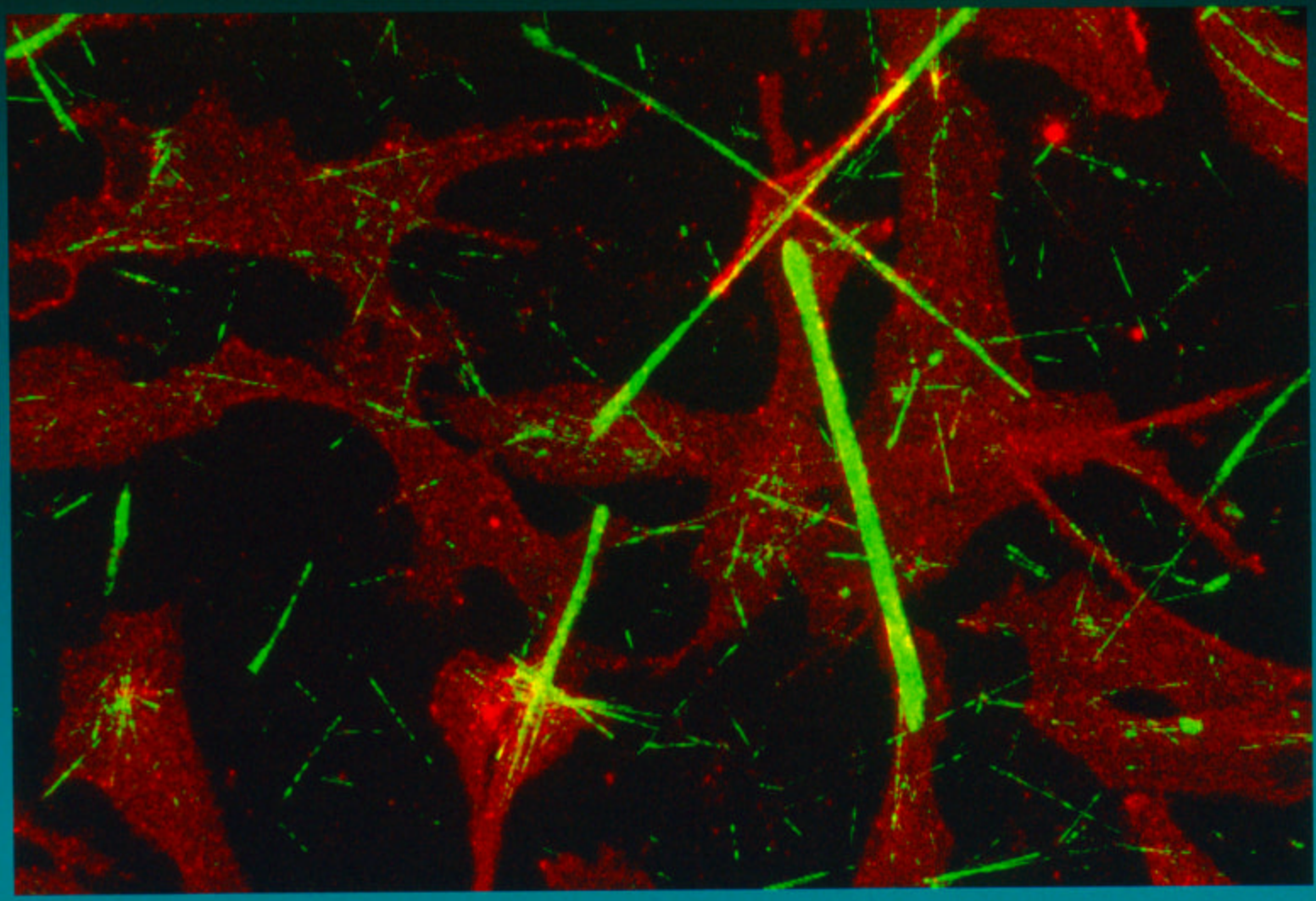


MAP KINASE ACTIVITY IN RAT PLEURAL MESOTHELIAL (RPM) CELLS

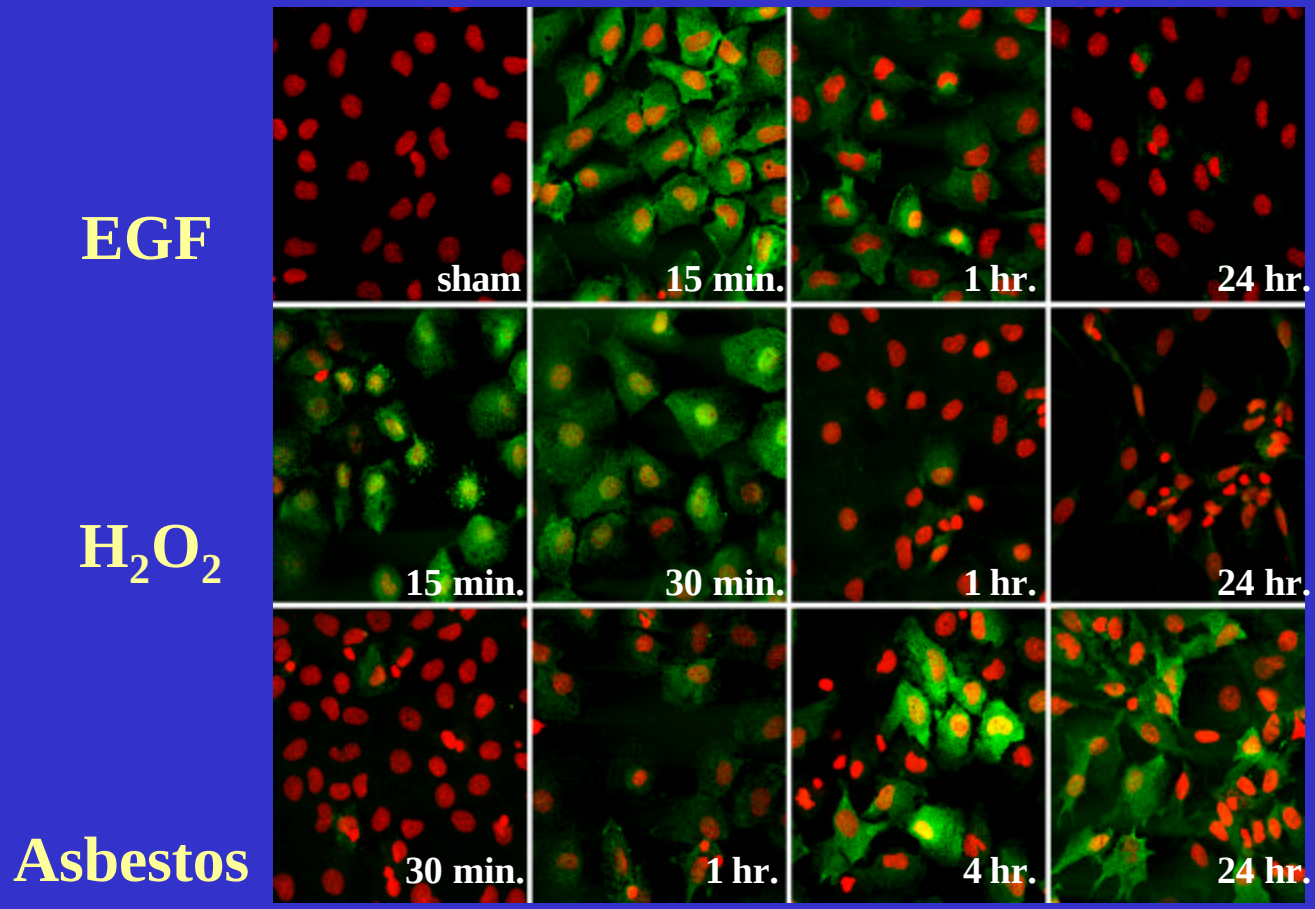



erk2 ACTIVITY IMMUNOPRECIPITATED FROM RAT PLEURAL MESOTHELIAL (RPM) CELLS
1 Hr.
8 Hr.
GST-myc (1-300)
0
EGF (5ng/ml)
0
EGF (5ng/ml)
Crocidolite (10μg/cm²)
1.25
2.5
5.0
10
25
Riebeckite (μg/cm²)
8 Hr.
GST-myc (1-300)
Chrysotile (10μg/cm²)
1.25
2.5
5.0
10
25
Antigorite (μg/cm²)
1.25
2.5
5.0
10
25
MMVF-10 (μg/cm²)

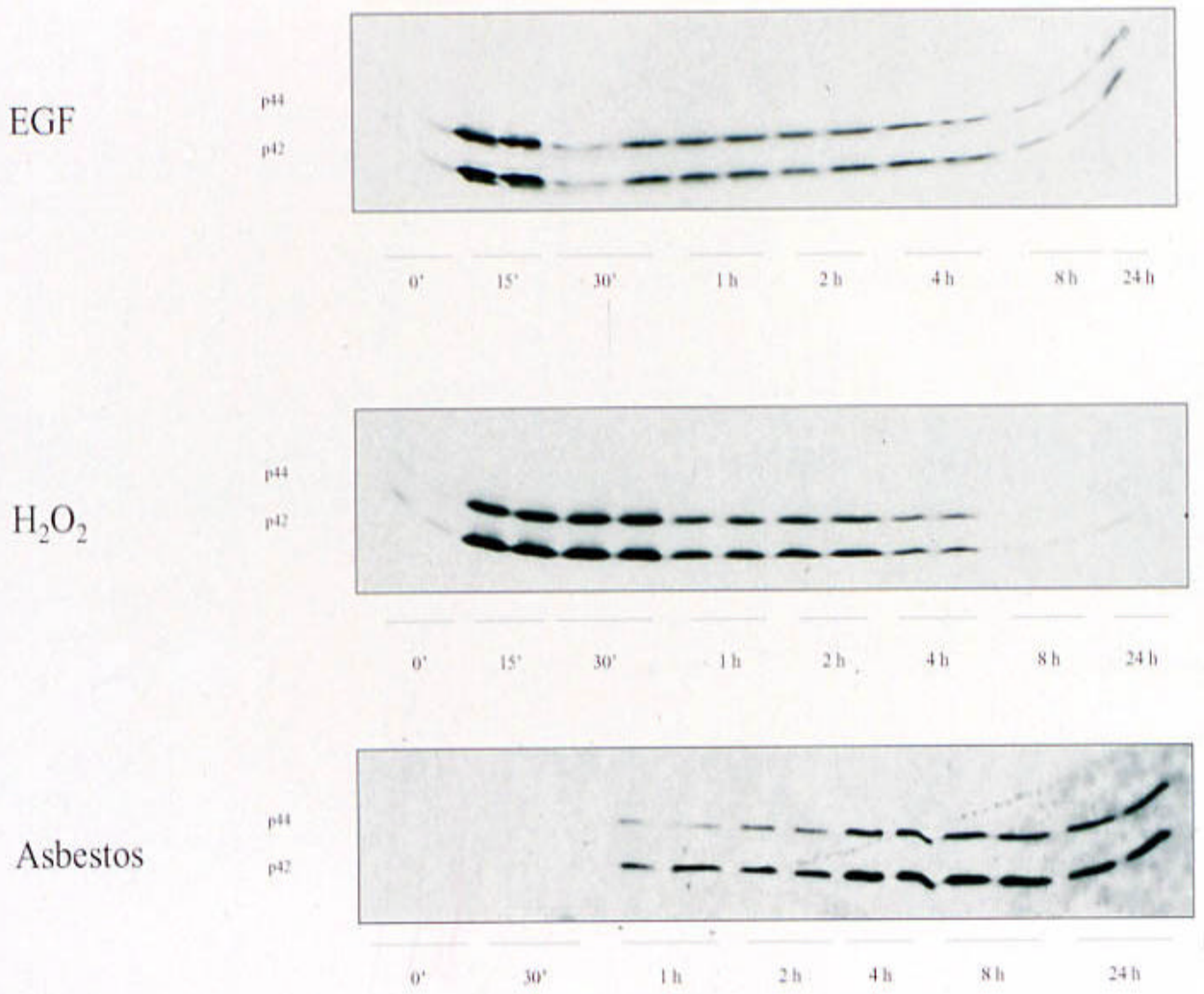
Human Met 5A Cells - Merged Confocal Images of EGF-R (Red) and Asbestos Fibers (Green)



Phospho-ERK in Lung Epithelial Cells

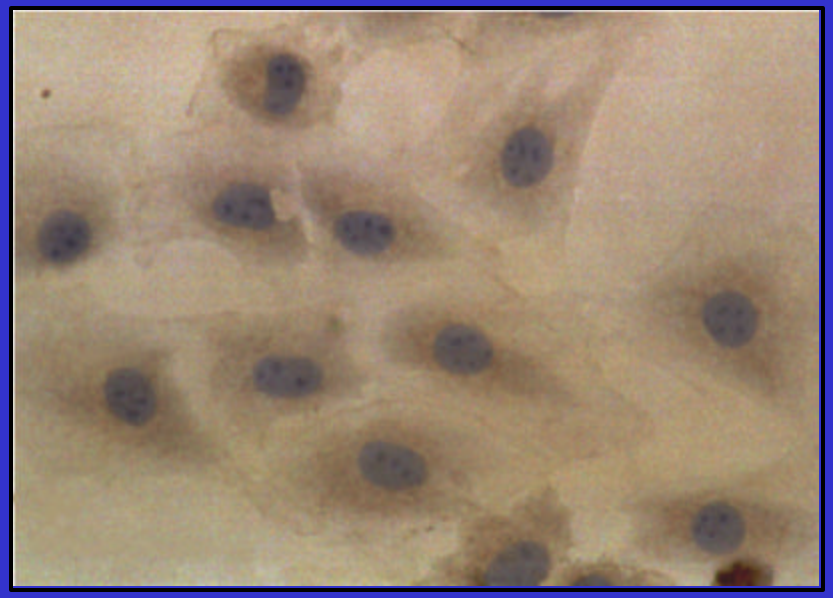


Western Blot for Phospho-ERKs

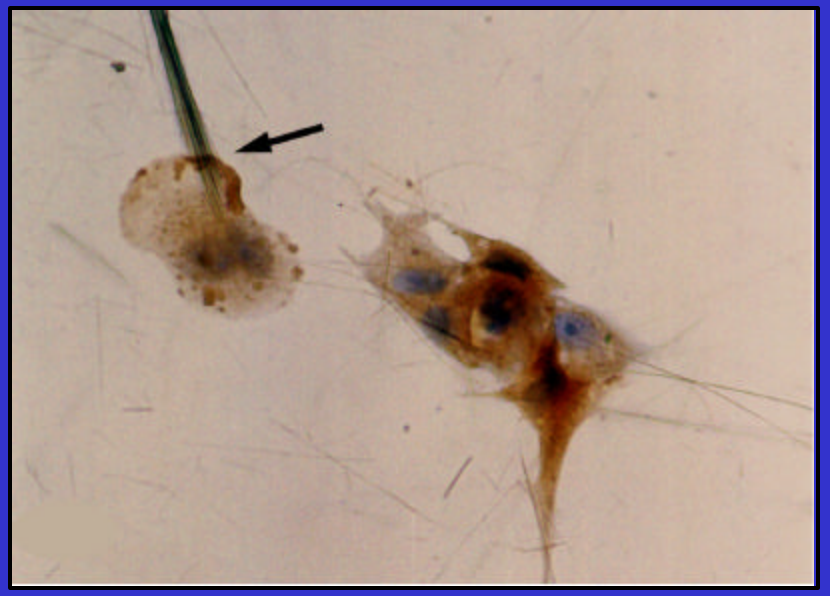


Immunoperoxidase Technique to Determine Changes in Cellular Distributions of Phosphorylated ERKs Following Exposure to Asbestos

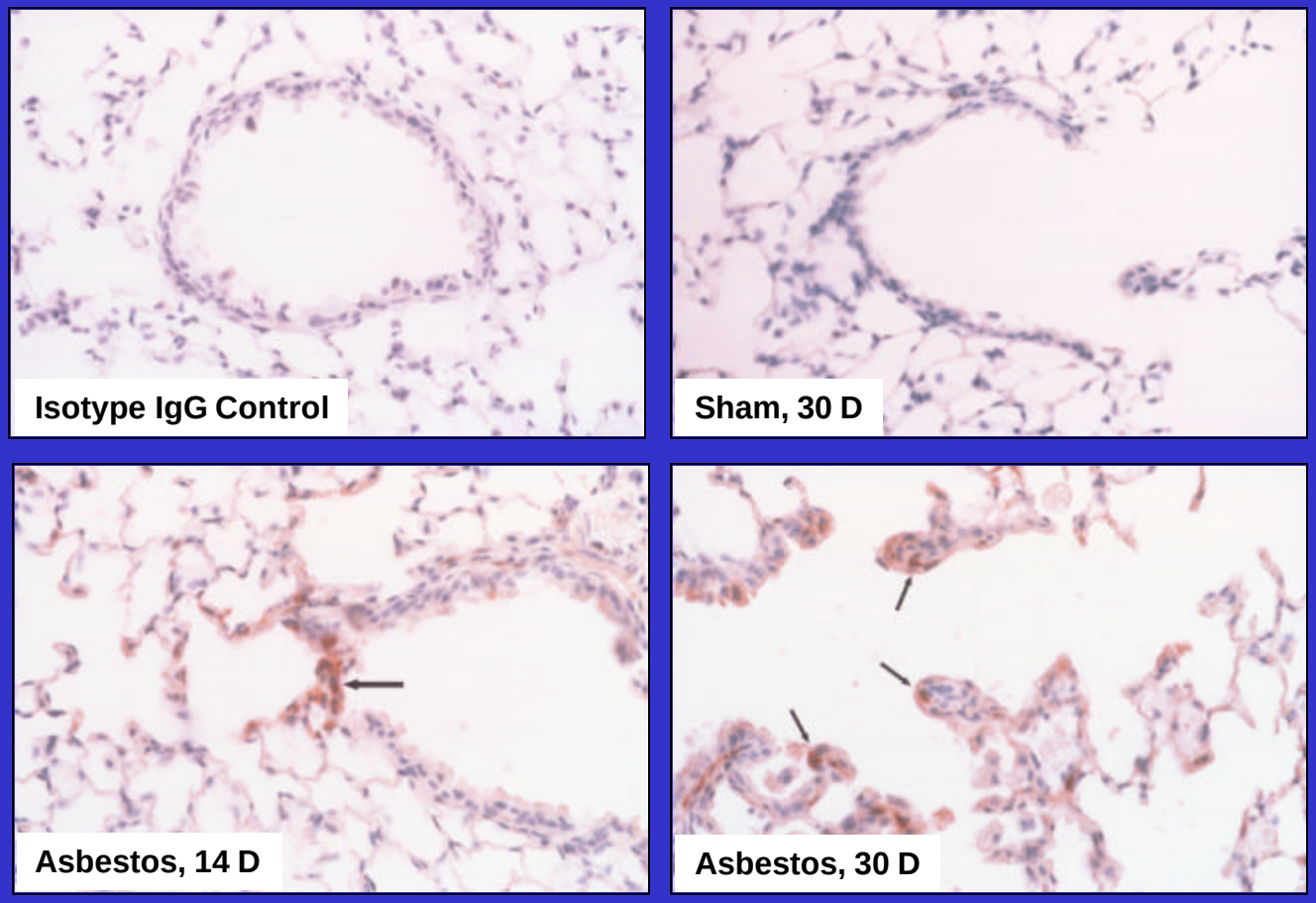
A. Control Cells



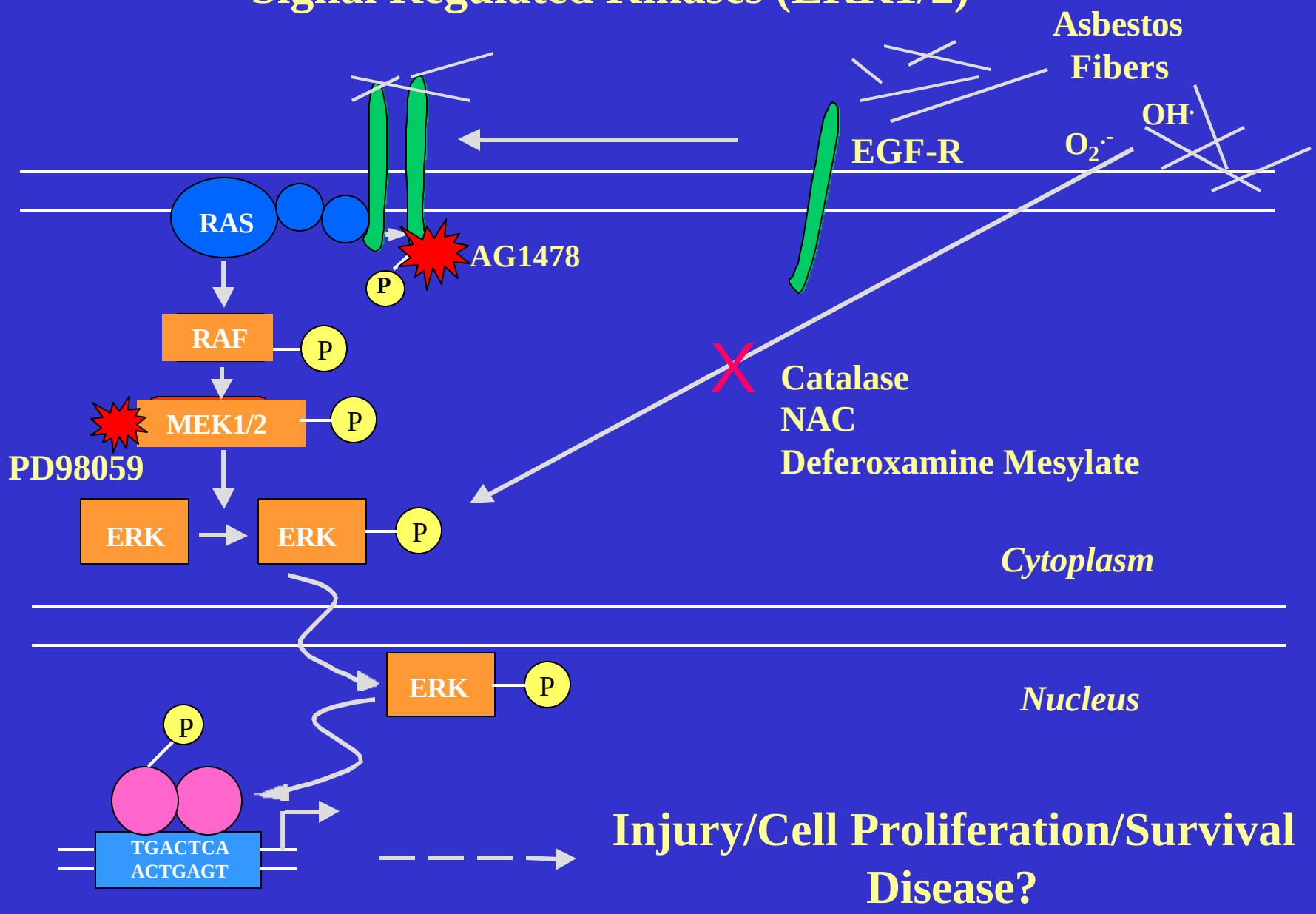
B. Asbestos-exposed Cells



Immunohistochemical Localization of ERK Phosphorylation



Asbestos-Induced Stimulation of Extracellular Signal Regulated Kinases (ERK1/2)



Summary

- **Cellular and molecular responses stimulated by asbestos fibers:**
  - **inflammatory response**
    - **generation of ROS/RNS by phagocytic cells**
    - **release of chemokines/ cytokines/ growth factors**
    - **cell injury/ cell proliferation**
      - ***in vivo* markers of cell proliferation**
      - ***in vitro* cell death (apoptosis), increases in total cell numbers**
  - **activation of cell signaling pathways, e.g. MAPK**
    - **interaction of asbestos fibers with growth factor receptors, e.g. EGFR**
    - **ERK phosphorylation by asbestos fibers but not nonpathogenic particles**

Summary cont.

- **translocation of phosphorylated ERK from cytoplasm to nucleus**
- **localization of phosphorylated ERK at sites of fiber deposition in lungs of animals exposed to crocidolite asbestos by inhalation**

– **transcription factor activation, e.g. AP-1**

- **increased expression of early response genes c-*fos* and c-*jun***
- **increased AP-1 DNA binding and transcriptional activity**